Mass Spectrometry in Biochemical Research*

By Einar Stenhagen

Institutionen för Medicinsk Biokemi, Universitet, Göteborg (Sweden)

"I have described at some length the application of Positive Rays to chemical analysis: one of the main reasons for writing this book was the hope that it might induce others, and especially chemists, to try this method of analysis. I feel sure that there are many problems in Chemistry which could be solved with far greater ease by this than by any other method. The method is surprisingly sensitive." J. J. Thomson, 1913

The first mass spectrometer was described in a book published in 1913[1]. The author was the famous British physicist J. J. Thomson, who at that time was Cavendish professor of physics at Cambridge. The citation above from the preface shows that the physicist Thomson clearly foresaw the importance of mass spectrometry in chemical analysis. To-day mass spectrometry is of very great importance in many branches of chemistry. The application of mass spectrometry to the structure determination of natural products began less than 15 years ago but it has, particularly during the last 5 years, grown to such an extent that on this occasion it is possible to deal only with a few selected topics.

If an organic compound is volatilized and submitted to electron bombardment at very low pressures the most common primary event is that a molecule loses an electron and is converted into positively charged molecule ion M^+.

$$M + e^- = M^+ + 2e^-$$

This is not stable and undergoes further decomposition into a number of fragments (charged and neutral). The pattern formed by the positively charged fragment ions is usually highly characteristic and can be used as a fingerprint of the molecule. The mass spectrometer sorts the ions according to their mass over charge (m/e) ratio and measures the intensities of the individual ion currents.

Some examples of studies of fragmentation mechanisms with the aid of heavy isotopes

It is evident that in order to identify the molecule by means of its mass spectrum it is important to know how the decomposition of the molecule ion M^+ takes place. Information about fragmentation mechanisms can often be obtained through studies of molecules labelled with heavy isotopes in specific positions. Our present knowledge is due to contributions from many workers.[2] Theory[3] is not yet advanced enough to permit the calculation of mass spectra of complex molecules.

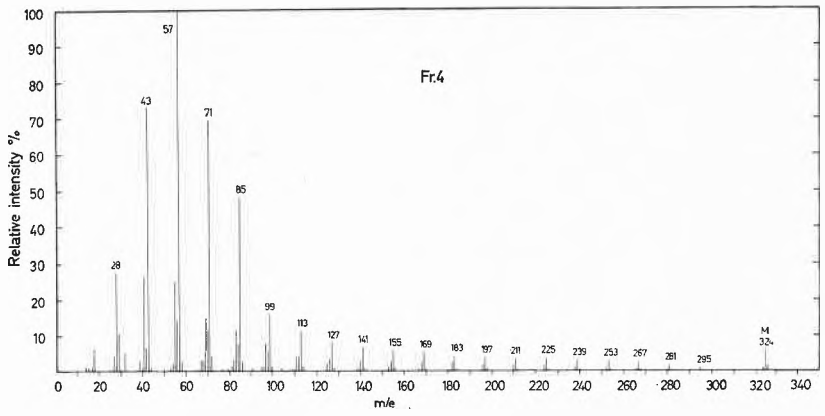


Fig. 1
Mass spectrum of *n*-tricosane. Electron energy 70 eV

Fig. 1 shows the mass spectrum of the normal chain hydrocarbon with 23 carbon atoms. At the far end of

* Lecture before the Chemical Society in Basel, June 23rd, 1966.

[1] J. J. Thomson, *Rays of Positive Electrocity and their Application to Chemical Analysis*, Longmans, Green, London 1913.

[2a] K. Biemann, *Mass Spectrometry. Organic Chemical Applications*, McGraw-Hill, New York 1962.

[2b] H. Budzikiewicz, C. Djerassi and D. H. Williams, *Interpretation of Mass Spectra of Organic Compounds*, Holden-Day, San Fransisco 1964.

[3] H. M. Rosenstock and M. Krauss, Quasi-Equilibrium Theory of Mass Spectra, article in *Mass Spectrometry of Organic Ions*, edited by F. W. McLafferty, Academic Press, New York/London, 1963, p. 1.

the spectrum we have the molecule-ion M^+ which gives the molecular weight directly. The mass spectrum is dominated by a series of ions of the empirical formula $[C_nH_{2n+1}]^+$ $(1 \leqq n \leqq 21)$. At first sight it appears that we have a series of ionized alkyl fragments formed through simple cleavage of the chain, the base peak at m/e 57 being due to butyl ions $[C_4H_9]^+$. If so, replacement of the terminal methyl groups by trideuteriomethyl groups might be expected to shift the base peak to m/e 60. The mass spectrum reproduced in Fig. 2 of an α,ω-di-(trideuteriomethyl)-substituted hydrocarbon shows a large peak at m/e 60 but the base peak remains at m/e 57. A series of "alkyl ions" that do not contain a trideuteriomethyl group extents up to $[C_{17}H_{35}]^+$, whereas the series of "normal" alkyl ions which arise from simple cleavage of the chain and in this case contains one trideuteriomethyl group, ends with the $[C_{21}H_{43}]^+$ member.

hydrocarbon chain, leading to formation of new carbon-carbon bonds and expulsion of the equivalents of alkyl groups. It is thus evident that the alkyl type ions found in the mass spectrum of a normal chain hydrocarbon are formed by (at least) three different mechanisms: (a) simple cleavage of the chain giving alkyl ions with one CD_3-group, (b) double cleavage with rearrangement of one hydrogen atom and (c) through expulsion of part of chain + one hydrogen atom, followed by coupling of the end fragments.

It is by no means certain that isobaric "alkyl" ions formed through different mechanism possess the same structure. For example, the ions of m/e 43 and m/e 57 in the mass spectrum of Fig. 2 may be, respectively, protonated and methylated cyclopropane structures,[5] whereas the ions of m/e 46 and 60 may be open chain alkyl ions.

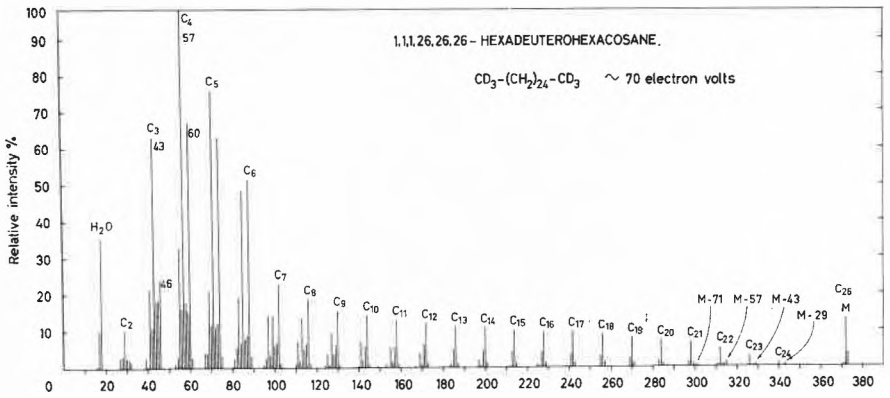


Fig. 2
Mass spectrum of 1,1,1,26,26,26-hexadeuteriohexacosane. From ref. [4]

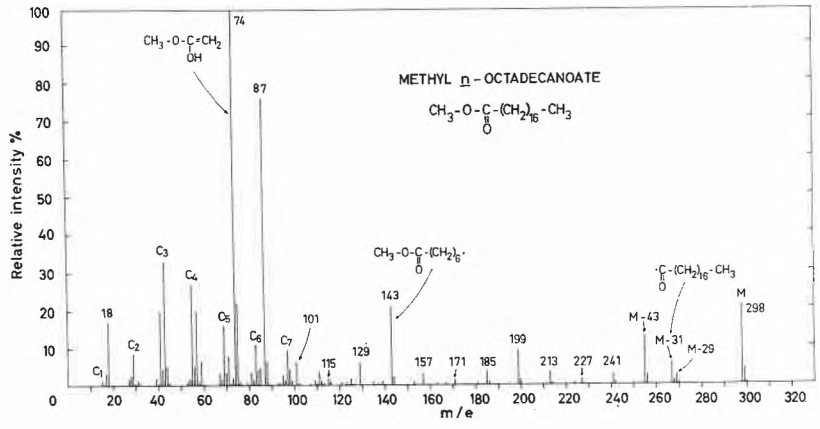


Fig. 3
Mass spectrum of methyl stearate. From ref. [11]

At the high mass end of the spectrum there are peaks at m/e = M-29, M-43, M-57 and M-71, the molecule-ion having lost the equivalent of a short nondeuterated alkyl group, both trideuteriomethyl end groups being retained in the fragment ion. These ions are probably the result of transhelical reactions in the randomly coiled

Studies of heavy atom labelled fatty acid esters gave information about the fragmentation modes of this class of compounds,[6] which made direct mass spectrometric

[4] Dinh-Nguyên, Nguyên, R. Ryhage, S. Ställberg-Stenhagen and E. Stenhagen, *Ark. Kemi 18* (1961) 393.

[5] cf. H. M. Grubb and S. Meyerson, Mass Spectra of Alkylbenzenes, article in *Mass Spectrometry of Organic Ions*, edited by F. W. Mc Lafferty, Academic Press, New York/London 1963, p. 453.

[6] R. Ryhage and E. Stenhagen, Mass Spectrometry of Long Chain Esters, in *Mass Spectrometry of Organic Ions*, edited by F. W. Mc Lafferty, Academic Press, New York/London 1963, p. 399.

structure determination possible for a number of compounds of biological interest.* The normal mass spectrum of methyl stearate obtained with bombarding electrons of 70 eV energy contains ions consisting both of hydrocarbon fragments and oxygen-containing fragments (Fig. 3). The former may be suppressed by lowering the electron energy to about 10 electron volt (Fig. 4). The series of ions of m/e 87, 101, 115 etc. could all arise through simple cleavage of the chain at position 3,4; 4,5; etc. but substitution of $-CD_3$ for the terminal methyl group (Fig. 5) shows that two different mechanisms operate a) simple cleavage of the chain and b) transhelical reactions involving the loss of the equivalent of an alkyl group from the chain, the end groups being retained in the ions.

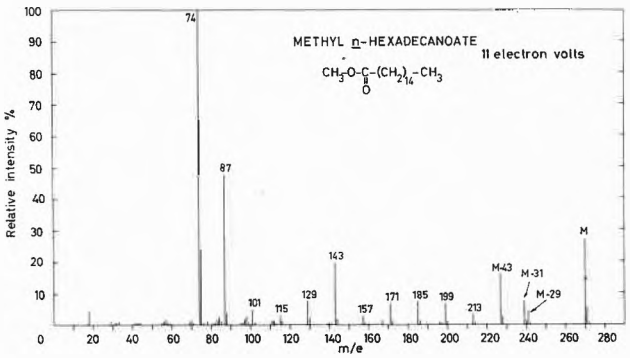


Fig. 4. Mass spectrum of methyl palmitate run at 11 eV. From ref. [11]

For example, detailed studies involving both heavy-atom labelled and methyl-substituted compounds (cf. ref. [6]) has shown that ions of m/e = M-43 in the mass spectrum of long chain esters arise through elimination of the methylene groups at position 2,3 and 4 together with one extra hydrogen atom. The mass spectrum of the 2-^{13}C-labelled ester (Fig. 6) shows a normal isotope peak (mostly due to the presence of about 1.1% ^{13}C in natural carbon) at m/e 256. If carbon atom 2 had been retained the peak at m/e 256 should have been higher than m/e 255, owing to the excess of ^{13}C of the carbon atoms in this position. That carbon atom 4 is also lost

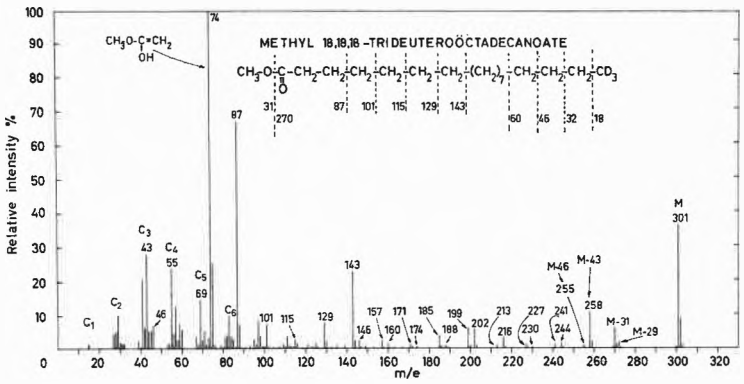


Fig. 5
Mass spectrum of ω-trideuterio-substituted methyl stearate. From ref. [4]

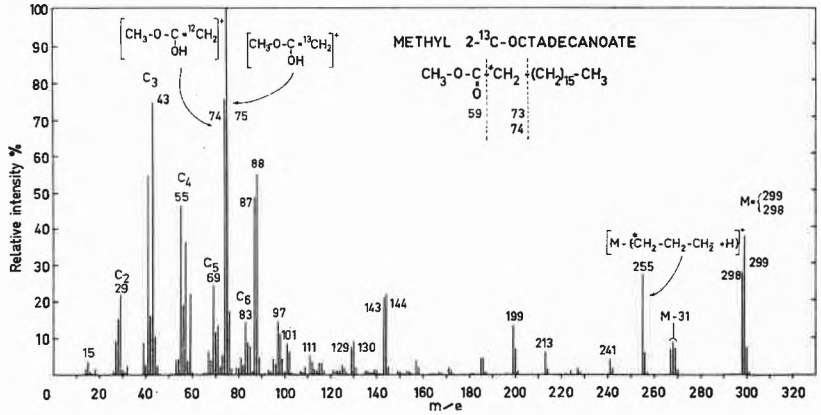


Fig. 6
Mass spectrum of methyl 2-^{13}C-octadecanoate. From ref. [4]

* For example, C_{32}-mycocerosic acid[7,8] and C_{27}-phthienoic acid[9,10] from the tubercle bacillus, phytanic acid from butter fat[11], and several branched chain acids present in the preen gland secretion of water fowls[12].

[7] C. Asselineau, J. Asselineau, R. Ryhage, S. Ställberg-Stenhagen and E. Stenhagen, *Acta Chem. Scand. 13* (1959) 822.

[8] R. Ryhage and E. Stenhagen, *J. Lipid Res. 1* (1960) 361.

[9] L. Ahlquist, C. Asselineau, J. Asselineau, S. Ställberg-Stenhagen and E. Stenhagen, *Ark. Kemi 13* (1958) 543.

[10] R. Ryhage, S. Ställberg-Stenhagen and E. Stenhagen, *Ark. Kemi 18* (1961) 179.

[11] E. Stenhagen, *Z. Anal. Chem. 181* (1961) 462.

[12] G. Odham, *Ark. Kemi 21* (1963) 379; *22* (1964) 417; *23* (1965) 431.

is shown by the peak at M-45 in the mass spectrum of a 4,4-dideuterio-substituted ester (Fig. 7).

The strongest peak in the methyl ester spectra shown in Figs 2–6 occurs at m/e 74 and is due to an ion formed through 2,3-cleavage and rearrangement of one hydrogen atom from the part of the molecule-ion lost. Systematic substitution of CD_2 for CH_2 at different positions along the chain has shown that the hydrogen atom is taken from position 4. The transfer of a deuterium atom leads a displacement of the base peak to m/e 75 for a 4,4-dideuterio-substituted methyl ester, as shown by Fig. 7.

For the 6,6-dideuterio-substituted esters (Fig. 8) the peak at m/e 88 is much higher than expected from the normal isotope effect. We have found that this is due to a very fast hydrogen-deuterium exchange between position 6 and position 2 prior to the cleavage of chain between carbon atoms 3 and 4. To a smaller extent this also occurs for the 5,5- (Fig. 8b) and 7,7-dideuterio-substituted esters,[4] and the phenomenon is obviously connected with the fact that carbon 2 can approach carbon atoms 5, 6 and 7 in space. These results show that in the interpretation of experimental data obtained with partially deuterated compounds false conclusions might be drawn if the possibility of fast deuterium-hydrogen exchange between carbon atoms that can approach each other in space is not taken into account.

In the course of work in the synthesis of organic compounds containing deuterium at specific positions we have found a catalytic system that is capable of ex-

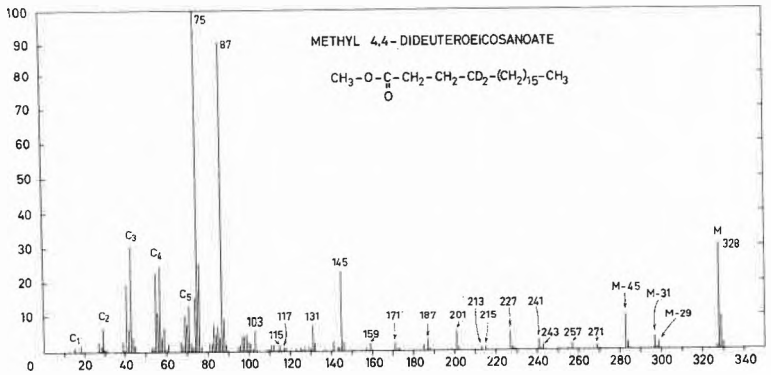


Fig. 7
Mass spectrum of 4,4-dideuterioeicosanoate. From ref. [4]

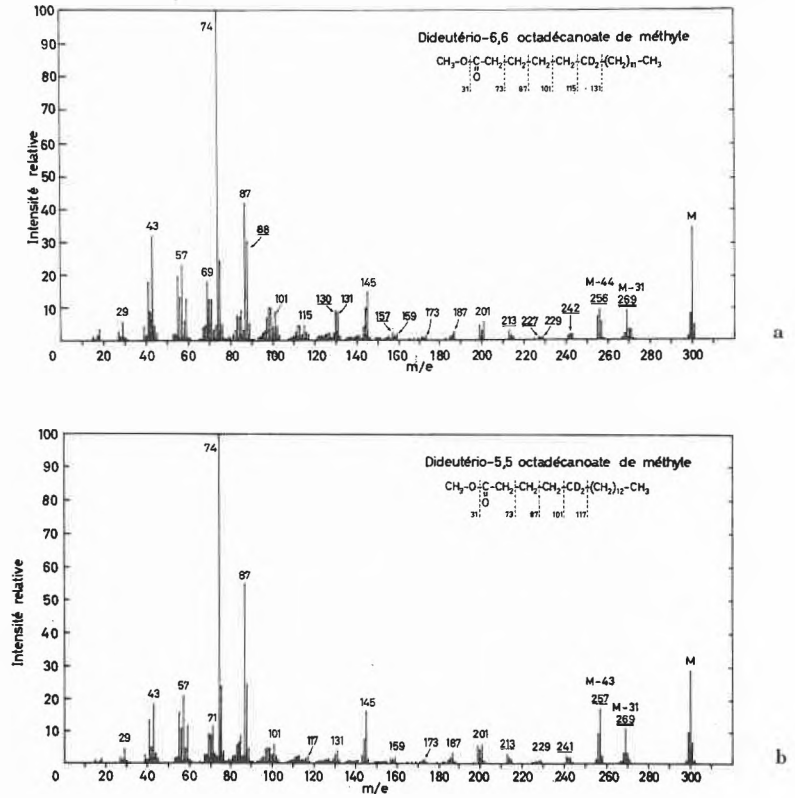


Fig. 8. Mass spectrum of
a) methyl 6,6-dideuteriostearate,
b) methyl 5,5-dideuteriostearate. From ref. [4]

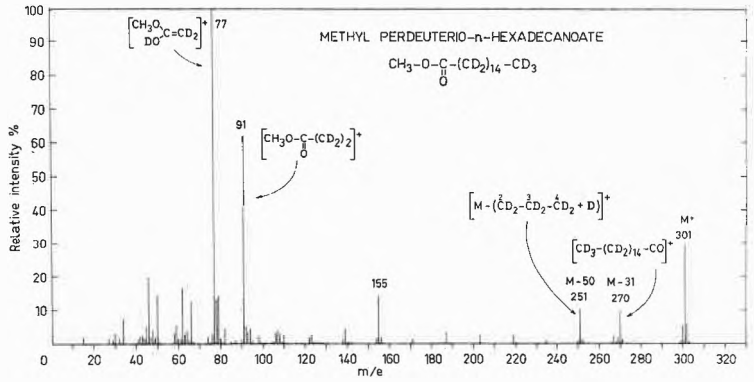


Fig. 9
Mass spectrum of methyl perdeuteriopalmitate

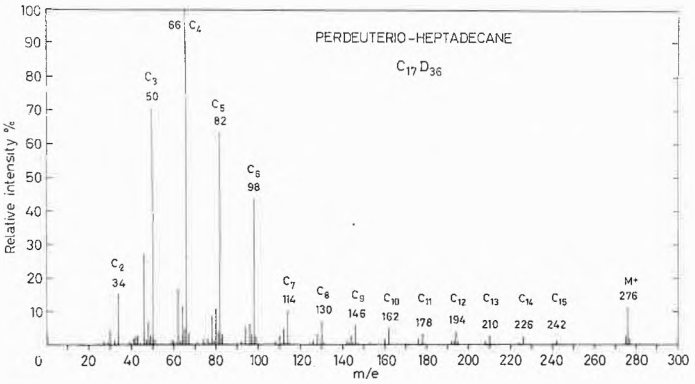


Fig. 10
Mass spectrum of perdeuterioheptadecane

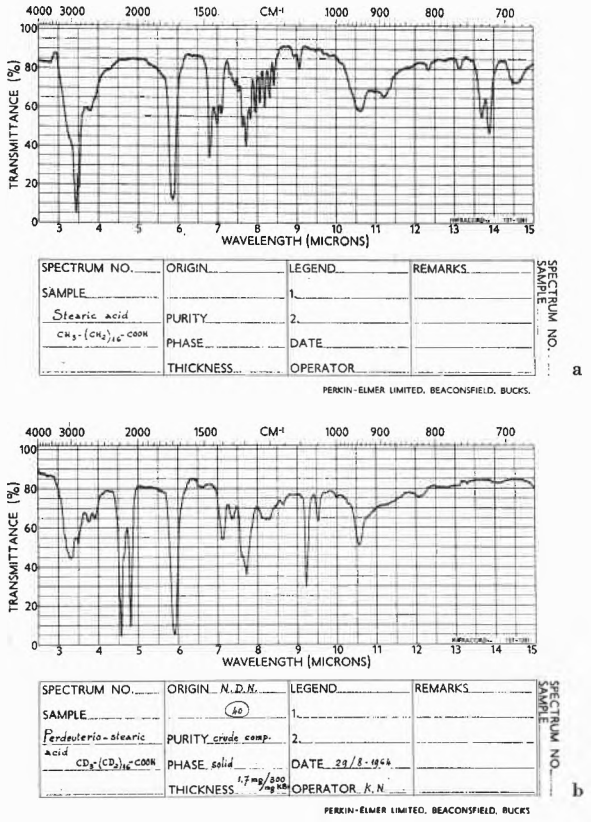


Fig. 11. Infrared absorption spectra in the solid state, a) stearic acid, b) perdeuteriostearic acid.

changing all hydrogen atoms for deuterium in large organic molecules[13, 14]. The mass spectrum of methyl perdeuteriohexadecanoate shown in Fig. 9 indicates an isotopic purity of about 99.2%. The spectrum, like that of the deuteriocarbon shown in Fig. 10, is quite analogous to that of the corresponding hydrogen compound. Fig. 11 shows the solid state IR-spectra of stearic acid and perdeuteriostearic acid respectively. The latter shows intense absorption in the 4.5–5 μ region where the hydrogen compound shows practically no absorption. It is now possible to start with fully deuterated compounds and label them with hydrogen at specific positions. Such "inverse" labelling may find interesting applications in biochemical tracer work as well as in spectroscopic work.

Examples of the use of directly combined gas chromatograph-mass spectrometer in biochemical research

There exists a wide variety of mass spectrometers, of which two types are of special importance in biochemical work: a) the double-focussing high-resolution type equipped with a direct inlet system and b) fast-scanning medium resolution types with a gas chromatograph as inlet system. High resolution organic mass spectrometry was

[13] DINH-NGUYÊN, NGUYÊN and E. STENHAGEN, Swedish Pat. Appl. 1280/1965.
[14] DINH-NGUYÊN, NGUYÊN and E. STENHAGEN, *Acta Chem. Scand.*, in the course of publication.

pioneered by BEYNON.[15] Very precise determinations of the mass of the ions (within a few millimass units) in many cases makes it possible to calculate the empirical formula of the ions, which is obviously of very great importance in determining the molecular structure of unknown compounds. Fig. 12 indicates the degree of resolution necessary to distinguish between a number of compounds of nominal mass 200 containing carbon, hydrogen, oxygen and nitrogen. Recent developments in high-resolution mass spectrometry have been discussed by MCLAFFERTY.[16]

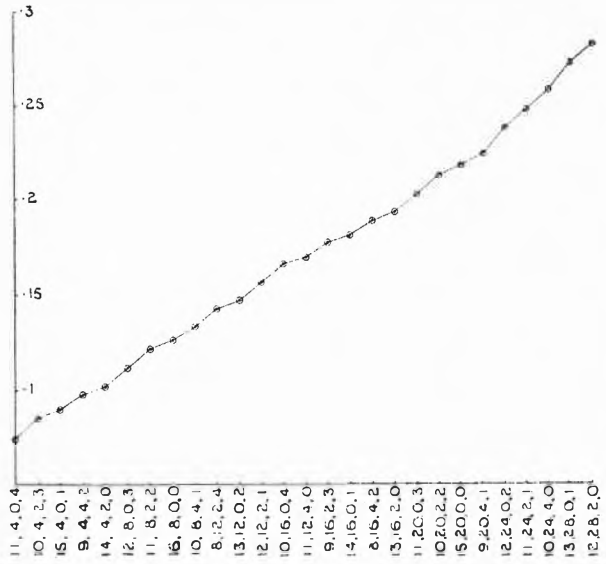


Fig. 12. Mass differences between various atomic combinations of carbon, hydrogen, nitrogen and oxygen of nominal mass 200. From ref. [15]

The direct combination of gas chromatograph and fast recording mass spectrometer of medium resolution (1:500 à 1:1500) is a most useful instrument in the biochemical laboratory. Once installed it will soon prove indispensible. The general arrangement of such an instrument is shown in Fig. 13. In order to retain the sensitivity of the mass spectrometer some means of selectively removing the transport gases must be used at least when compounds of high molecular weight are analyzed. The instrument in daily use in our laboratory[17] is equipped with a BECKER jet[18] for this purpose.

Furthermore, it is very important, in order to avoid excessive background, to use stationary phases which do not bleed appreciably at the operating temperature. It is not possible simply to substract the background obtained between the gas chromatographic peaks because it is found that the background *increases* during the elution of the gas chromatographic fractions, presumably because of displacement of volatile stationary phase material by the separated fractions. This effect, which seems to have been overlooked by other workers, cf. ref.[19], is quite pronounced in high temperature work with compounds of high molecular weight.

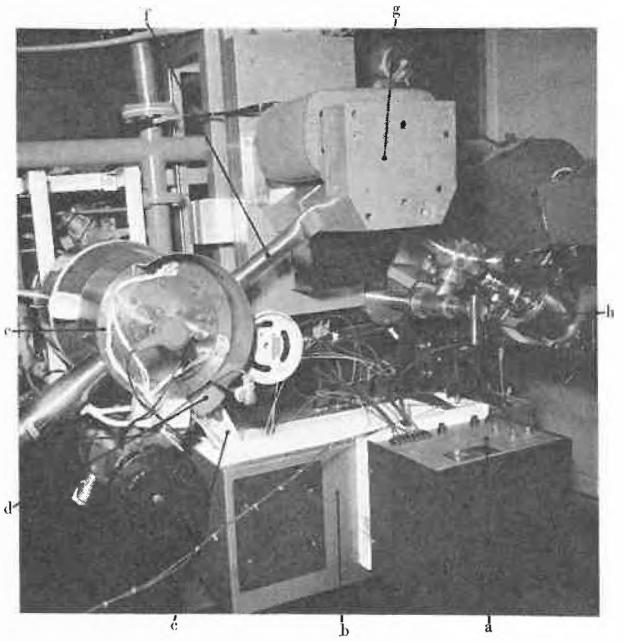


Fig. 13. Top view of a gas chromatograph-mass spectrometer (GC-MS) combination. a) temperature programmer, b) gas chromatograph with flame ionization detector connected in parallel with the mass spectrometer, c) heated line between gas chromatograph and jet separator, d) jet separator, e) ion source housing, f) analyzer tube, g) magnet, h) collector (electron multiplier)

As an example of work performed with the combined instrument I will take the analysis of the scent of the two bees *Halictus calceatus* and *Halictus albipes* carried out last year in our laboratory.[20] These are small solitary bees which to a sensitive human nose have a musk-like odour. A modified "enfleurage" technique was used in which the volatile material from the animal was taken up by stationary phase material (silicone high vacuum grease). The absorbed material was driven out by heating under reduced pressure, condensed in a small cold trap, taken up in a small volume of solvent and the resulting solution injected into the gas chromatograph. Fig. 14 shows the gas chromatogram. A comparison between the responses of the hydrogen flame detector and the total ionization monitor showed that fraction 2 and 3 were silicon compounds derived from the stationary phase. Fraction 4 was directly identified as *n*-tricosane from the mass spectrum shown in Fig. 1. Odd numbered hydrocarbons of this type are always present on the surfaces of insects and plants. Fraction 6 was identified as the saturated ring lactone dihydroambrett-

[15] J.H. BEYNON in *Advances in Mass Spectrometry*, edited by J. D. WALDRON, Pergamon Press, London 1959, p. 328.
[16] F.W. MCLAFFERTY, *Science 151* (1966) 641.
[17] cf. E. STENHAGEN, *Z. anal. Chem. 205* (1964) 109.
[18] E.W. BECKER, K. BIER, H. BURGHOFF and F. ZIGAN, *Z. Naturforsch. 12a* (1957) 609.
[19] J.T. WATSON and K. BIEMANN, *Anal. Chem. 37* (1965) 844.
[20] C.-O. ANDERSSON, G. BERGSTRÖM, B. KULLENBERG and S. STÄLLBERG-STENHAGEN, *Ark. Kemi*, in the course of publication.

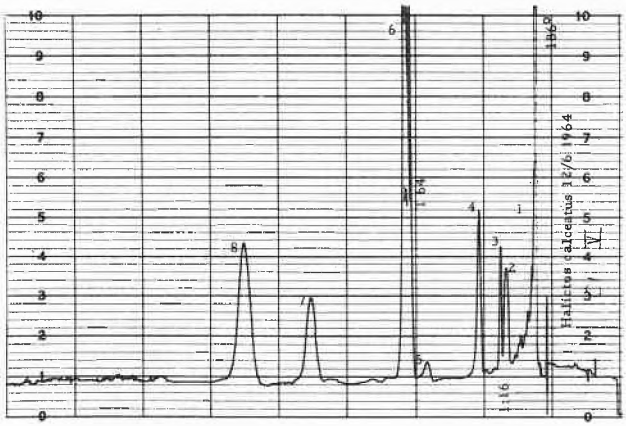


Fig. 14. Gas chromatogram of volatile scent material from *Halictus calceatus*. GC-MS combination with hyprose as stationary phase at 186°. From ref. [20]

olide by comparison with the mass spectrum of authentic material (Figs 15 and 16). Fraction 7 was identified as the C_{18} homologue of dihydroambrettolide and fraction 8 as a mono-unsaturated ring lactone with 18 carbon atoms (Fig. 17). The analysis of the *Halictus albipes* sp. was carried out using a total of 9 animals. BUTENANDT and co-workers [21] in their famous work on the sex attractant of the silk moth, *Bombyx mori*, used half a million glands in the final analysis. JONES, JACOBSON and MARTIN [22] in analogous work on the sex attractant of the pink bollworm moth reports the use of 500.000 animals. A strict comparison is of course not possible and the work just mentioned may have been much more difficult, but the

[21] A. BUTENANDT, R. BECKMANN, D. STAMM and E. HECKER, *Z. Naturforsch. 14b* (1959) 283.

[22] W. A. JONES, M. JACOBSON and D. F. MARTIN, *Science 152* (1966) 15.

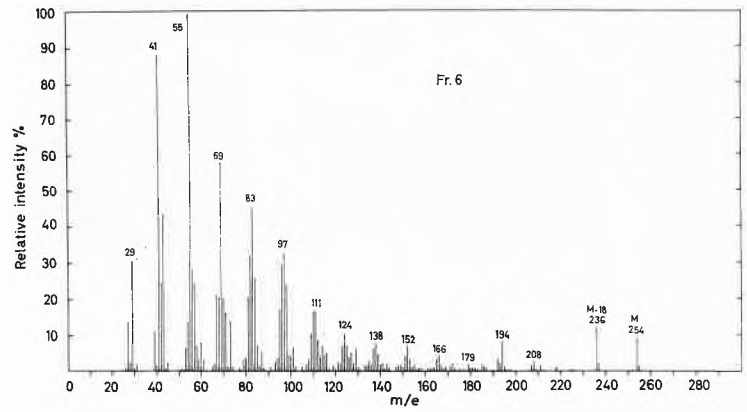


Fig. 15
Mass spectrum of fraction 6 in the gas chromatogram shown in Fig. 14. Recording time one second

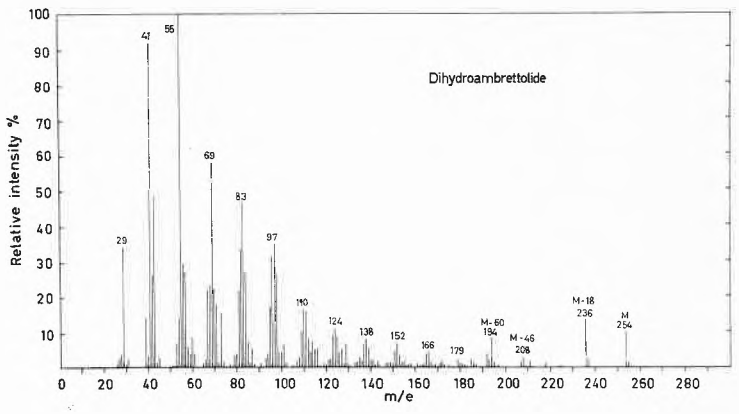


Fig. 16
Mass spectrum of dihydroambrettolide prepared by hydrogenating, ambrettolide (Firmenich) over platinum oxide catalyst. From ref. [20]

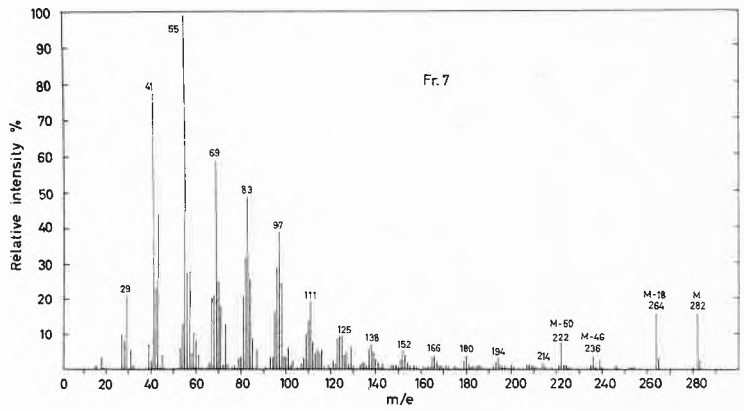


Fig. 17
Mass spectrum of fraction 6 (Fig. 14). From ref. [20]

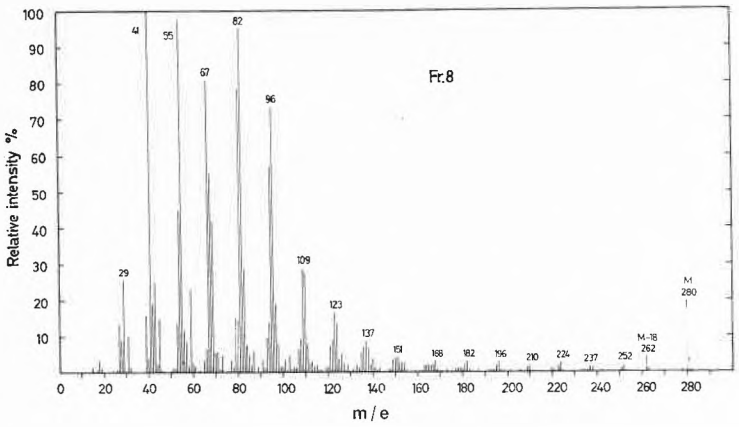


Fig. 18
Mass spectrum of fraction 8 (Fig. 14).
From ref. [20]

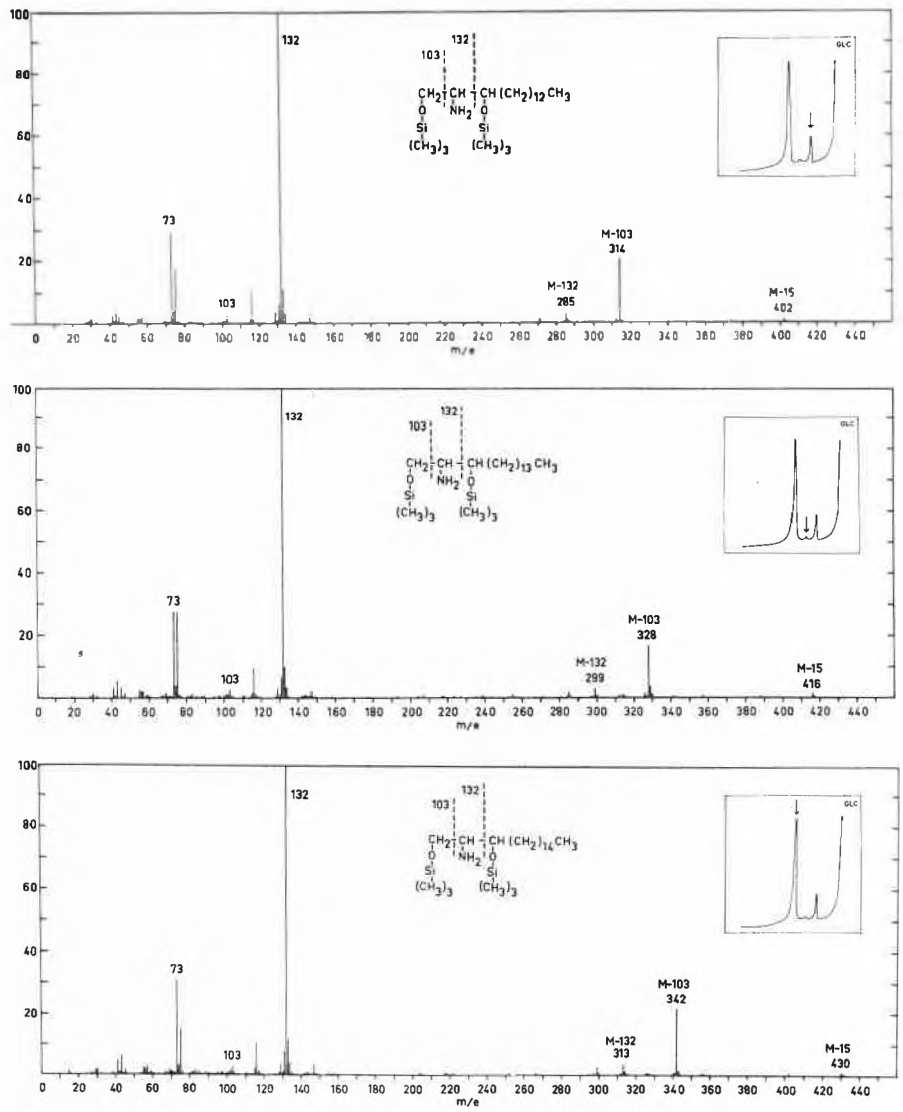


Fig. 19
GC-MS analysis of mixture of dihydrosphingosines (as di-trimethylsilyl ethers), cf. ref. [21]

figures mentioned may be used to stress the power of the newer technique.

If the compounds studied are not themselves volatile enough for analysis it is often possible to convert them into more volatile derivatives. Fig. 19 shows an analysis of material isolated from the sphingomyelin fraction of human blood serum, carried out by K. A. KARLSSON in our laboratory. After hydrolysis, hydrogenation and conversion of the hydroxyl groups into trimethylsilyl ether groups,[23] the material was separated into three

[23] cf. K.-A. KARLSSON, *Acta Chem. Scand. 19* (1965) 2425.

fractions which could be directly identified by means of the mass spectra as C_{16}, C_{17} and C_{18} dihydrosphingosines.

The data retrieval problem in mass spectrometry

We are at present working on the marking perfumes of bumble bees. The male bumble bee has the habit of marking out its territory by depositing a volatile scent material present in the secretion of the mandibular glands on leaves and stems of trees and plants. These markings have a very pleasant odour as perceived by the human nose. The scent material from the mandibular glands from males of 9 different species of bumble bees have been studied and the composition of the scent has been found to be quite characteristic for each species. A large number of excellent mass spectra have been obtained indicating the presence of sesquiterpene alcohols and some macrocyclic compounds, but in several cases we have not yet been able to identify the compound from the mass spectra.

This brings up the problem of identifying and sorting mass spectra. There is at present no means available to find out whether the mass spectrum of a certain compound has been previously recorded in the literature, and much of the material is present in collections such as the ASTM (American Society for Testing Materials) "uncertified mass spectra" and the API (American Petroleum Institute) collection, which are not generally available.

At the suggestion of professor ABRAHAMSSON in our laboratory, who is an X-Ray crystallographer and an expert in computer technique, a start was made some time ago (cf. ref. [17]) to work out a computer-based information retrieval system for organic mass spectrometry. We were fortunate to get the support of the Swedish Office of Organization and Management of the Swedish Finance Department in the formidable task of transferring tabular material for som 7000 mass spectra which we had available to magnetic tape (where the information occcupies less than one reel). A set of computer programs have been worked out for using the data in various ways. The computer is a Datasaab D21 which we have available in the laboratory. The computer-based mass spectrometric data retrieval system is very flexible and it shows a very satisfactory performance[24]. Alfabetical indexes or indexes after molecular weight can be produced. The computer can be ordered to write out any spectrum present in the tape memory in tabular form as well as in the form of a line diagram on the attached x,y-recorder or on the line printer (cf. Fig. 20). Several different keys are provided which are used for fast search through the file. Fig. 21 shows the result of searching through the data with 2-methylnaphthalene as "unknown" spectrum using a key of the 5 strongest lines. The figure in the last column represents a reliability index which is proportional to the sum of the differences formed when the spectra are compared line for line. It is possible to search with keys requiring certain intensity conditions to be fulfilled. Weighted keys can also be used, and the search can be performed within specified m/e ranges.

The usefulness and the importance of a data retrieval system of this type increases of course with the amount of information it contains.*

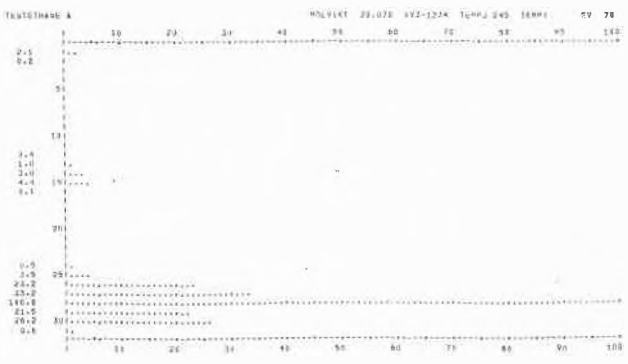


Fig. 20. Line printer print out of ethane mass spectrum. The accurate intensity figure is printed below the peak

RESULT.AFTER.COMPARE.AND.SORT

MOLVIKT	TITEL	BETECKN.	ANT	NR	HRK
142.000	2-METHYLNAPHTHALENE	DOW 2579	92	1	0
142.190	2-METHYLNAPHTHALENE (GAS)	API 981	114	1	1
142.190	2-METHYLNAPHTHALENE (GAS)	API 655	126	1	2
142.200	2-METHYLNAPHTHALENE (GAS)	API 894	120	1	2
142.190	2-METHYLNAPHTHALENE (GAS)	API 1015	134	1	2
142.198	1-METHYLNAPHTHALENE (GAS)	API 990	118	1	2
142.200	1-METHYLNAPHTHALENE (GAS)	API 893	132	1	2
174.000	1-(CHLOROMETHYL) NAPHTALENE	DOW 2957	58	1	5
142.000	1-METHYLNAPHTHALENE	DOW 352	52	1	8
170.000	PHENYL ETHER	DOW 770	80	1	9
186.000	DODECETIC ACID	DOW 3299	72	1	11
142.190	1-METHYLNAPHTHALENE (GAS)	API 487	41	1	11
198.000	9(OR10)9,9(11),3,9-TRIOXAUNDECANE (+01	ARC 105	7	1	12
184.270	2-N-BUTYLNAPHTHALENE (GAS)	API 1016	163	1	13
142.000	6-CHLORO-O-CRESOL	DOW 1549	111	1	13
156.088	1-ETHYLNAPHTHALENE	DOW 4118	126	1	13
142.000	BROMO-1,2-DIFLUOROETHYLENE	DOW 1211	66	1	13
184.280	2-N-BUTYLNAPHTHALENE	API 1518	169	1	14
169.030	2-CHLORO-P-XYLENE	DOW 2195	94	1	16
143.180	6-METHYLQUINOLINE	API 1562	142	1	16
158.000	1-METHOXYNAPHTHALENE	DOW 1447	98	1	16
140.000	4-CHLORO-O-XYLENE	DOW 2764	108	1	16
184.000	6-CHLORO-O-TOLYL ACETATE	DOW 3659	94	1	16
148.000	-DIFLUOROSTYRENE	DOW 2203	85	1	16
140.680	4-CHLORO-M-XYLENE	DOW 2821	92	1	17
142.000	IODOMETHANE	DOW 465	36	1	17
142.000	4-CHLORO-M-CRESOL	DOW 4598	104	1	17
145.000	2,5-DIMETHYL INDOLE	IC ja	95	1	17
157.210	2,6-DIMETHYLQUINOLINE (GAS)	API 621	148	1	17
140.000	O-CHLOROBENZALDEHYDE	DOW 2053	88	1	17
124.900	1-ETHYL-4-FLUOROBENZENE	DOW 461	110	1	17
141.950	IODOMETHANE (GAS)	API 696	15	1	17
140.000	P-CHLOROBENZALDEHYDE	DOW 4802	96	1	19
186.000	2-(4-CHLORO-2-METHYLPHENOXY)ETHANOL	DOW 3478	139	1	19
156.000	1(2-CHLOROPHENYL) ETHANOL	DOW 1362	133	1	20
140.000	M-CHLOROBENZALDEHYDE	DOW 4801	136	1	23
178.000	1,2,3,4-TETRAHYDROCARBAZOLE	SWA 10	126	1	26
140.000	1-CHLORO-3-ETHYLBENZENE	DOW 1782	134	1	21
142.000	4-CHLORO-O-CRESOL	DOW 2086	123	1	21
155.000	2-(O-CHLOROPHENYL)ETHYLAMINE	DOW 896	131	1	21
140.000	1-BROMO-2-CHLOROETHYLENE	DOW 460	6	1	21
140.000	O-CHLOROBENZYL ALCOHOL	DOW 3502	110	1	22
184.000	2-TERT-BUTYL-6-CHLOROPHENOL	DOW 2327	153	1	22
142.000	1-CHLORO-2-BROMOETHANE	DOW 464	78	1	24
176.000	4,4-DICHLORO-O-CRESOL	DOW 2574	156	1	27
140.000	ORTHOCHLOROBENZALDEHYDE	CCC 31	115	1	30
142.000	BROMOPENTABORANE	CCC 30	148	1	99

Fig. 21. Line printer print out of the result of a search, using the DOW 2579 spectrum of 2-methylnaphthalene as the spectrum of an unknown compound. The identity with the same spectrum in the file on magnetic tape is indicated by the index mark zero. Minor differences with the other API spectra of the same compound are indicated by index marks different from zero. Several of the figures for the molecular weight are nominal figures only. No restriction as to molecular weight was applied in the search

* The question how the information now on magnetic tape and the program system shall be made generally available is at present under consideration by a group formed at the recent ASTM committee E-14 mass spectrometry meeting in Dallas (Texas).

[24] S. ABRAHAMSSON, G. HÄGGSTRÖM and E. STENHAGEN, ASTM *Committee E-14 meeting on Mass Spectrometry, Dallas (Texas) 1966.*

Mass spectra obtained through charge exchange
Chemical ionization mass spectra

All the mass spectra discussed so far have been obtained through electron impact. The energy of the bombarding electrons is usually much higher than the energy required to produce the molecule-ion. Some of the excess energy is carried off by the electron that comes off in the ionization but the actual amount of energy imparted to the molecule ist not known. If molecules are bombarded with positive ions instead of electrons, ionization can take place by means of charge exchange. The energy transferred is then equal to the recombination energy of the ions used for bombardment, and this energy is usually well known. Charge exchange mass spectra have been studied by several authors, but perhaps most extensively by LINDHOLM and co-workers in Stockholm. LINDHOLM uses a double mass spectrometer as shown in Fig. 22. Mass spectrometer A produces ions of the desired kind whose kinetic energy may be controlled by a series of retarding slits. Ionization by charge exchange with the molecules of the gas under study occurs in the collision chamber. The resulting mass spectrum is recorded by mass spectrometer B. Table 1 shows some of the results for ethanol obtained by VON KOCH and LINDHOLM.[25] The relative amount of the various ions in the mass spectrum varies considerably with the recombination energy of the incident ion. For instance, the

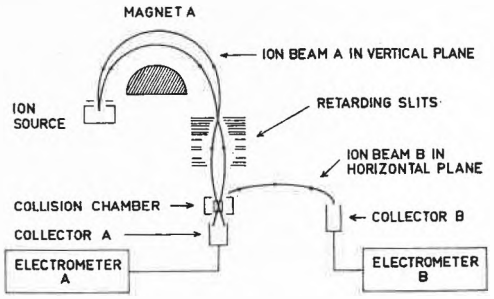


Fig. 22. Arrangement for measuring mass spectra produced through charge exchange. From ref. [25]

relative intensity of the molecule-ion of m/e 46 varies from 0.1 per cent for He^+ to 58.8 per cent for $[H_2S]^+$ as incident ion. Fig. 23 shows in graphical form the variation in the abundance of different ions in the mass spectrum of ethanol as a function of absorbed energy. It is evident that LINDHOLM's method, involving a specific transfer of energy, is capable of yielding much important information.

Fig. 24 shows the structures deduced by VON KOCH and LINDHOLM for a number of ions in the mass spectrum of ethanol. It may be pointed out in this connexion that so far all evidence for structures of organic ions

[25] H. VON KOCH and E. LINDHOLM, *Ark. Fysik 19* (1961) 123.

Table 1. Data taken from ref. [25] showing the variation of the mass spectrum of ethanol in charge exchange ionization with different incident ions

Incident ion	Gas	KE eV	46	45	44	43	42	41	31	30	29	28	27	26	25	19	17	16	15	14	13	12	47	18
H^+	H_2	5	1.3	25.0	0.2	1.9	<0.2		54.9	2.7	5.5	1.6	3.0	<0.7		2.2			0.7				3.8	
H^+		15	0.9	21.5		2.1			59.0	2.7	6.0	1.7	2.7	0.2		2.4			0.9				2.1	0.3
H^+		30	3.8	23.0	0.3	1.8	0.2		58.5	2.6	5.0	1.7	2.3	0.3		2.6			0.8				2.6	0.5
He^+	He	22	0.1	0.6	0.2	5.0	11.0	1.1	4.2	1.9	23.1	1.8	14.7	9.4	0.3	0.9		0.5	17.3	7.2	0.7		0.5	0.3
He^{++}	He	15	8.5	23.4		2.4			50.2	2.3	4.1	1.5	4.3	0.4		2.7			0.5				2.4	3
He^{++}		30	8.3	24.0	0.4	2.2			49.3	2.5	4.3	1.4	4.5	0.4		1.8			0.9				1.0	4
He^{++}		300	13.2	24.6	0.3	2.0	0.1		45.7	2.3	4.0	1.2	3.8	0.5		1.6			0.6				2.2	3
B^+	BF_3	11	0.4	29.0	0.8	1.6			54.0	2.7	4.5	1.4	3.2			1.7			0.9				2.2	1.0
B^+		30	0.7	29.0	0.7	2.2			54.0	2.7	3.8	1.2	2.7			1.8			0.9				3.2	0.6
B^+		900	6.0	25.5	0.9	2.0			51.0	3.3	3.8	1.3	2.5	0.5		1.3			1.5				3.3	0.5
C^+	CO	30	6.8	50.4	0.4	2.5	<0.2		28.2	1.0	3.3	0.6	3.5	0.6		1.3			1.3				3.8	
C^+		900	32.9	35.7	0.7	1.4	<0.2		21.2	1.1	2.1	0.4	2.5	0.4		0.6			0.9	0.2			4.0	0.2
C^+	CO_2	30	7.4	52.1		2.6			28.4	1.0	3.3	0.6	2.8			1.1			0.8				5.3	
C^+		900	34.0	35.8		0.9			22.5	0.9	2.7	0.2	2.1			0.4			0.4				5.4	
C^+	COS	22	6.2	53.4		1.9			30.9	0.5	2.9	0.1	2.7			0.8			0.5				3.7	
CH^+	CH_4	30	41.2	36.0	0.8	1.3			14.7	0.4	2.0	0.3	1.6	0.04		1.0			0.4				4.5	
CH_2^+	CH_4	15	37.2	37.1	0.6	1.5			10.8	0.4	4.5	0.6	3.9			2.9			0.6				4.8	
CH_3^+	CH_4	15	6.0	55.1		3.0			12.3	0.6	7.6	1.0	7.6			5.6			1.3				3.0	
CH_4^+	CH_4	15	26.6	34.5	0.3	0.1			26.7	0.6	7.4	0.2	0.6			2.6			0.3				4.6	
CO^+	CO	11	<0.4	12.5	<0.4	1.3			64.3	3.2	8.0	2.6	1.6			5.1			<0.4				1.6	<0.4
CO^+	CO_2	15	1.2	12.3	0.5	2.5			56.2	2.5	9.8	2.8	4.8	0.6		6.2			0.6				3.1	
CO^+	COS	15	0.3	13.5		1.3			64.2	2.6	9.0	2.9	2.2			4.2							2.2	
N_2^+	N_2	15	2.0	2.4	0.2	7.5	<0.2		40.8	1.4	10.4	3.1	23.2	1.6		6.1			1.1				1.4	2
N_2^+		900	1.8	5.8		10.1			36.2	2.0	11.9	2.7	19.0	2.5		4.2			3.1	0.7			1.8	
NO^+	N_2O	30	18.5	24.7	0.6	2.7			34.1	1.7	6.1	1.5	6.3	0.6		1.9			1.4				4.6	1
HS^+	H_2S	30	39.9	24.4					28.1		3.8		2.4			1.4							7.8	
H_2S^+	H_2S	30	58.8	20.0		0.2			15.3		2.9		1.1			1.6							9.4	
CO_2^+	CO_2	30	0.7	16.2		1.0			67.6	2.4	6.4	2.4	1.4			2.0							2.7	0.5
N_2O^+	N_2O	22	0.6	19.5		0.3			70.9	2.5	3.2	1.1	0.9			1.1							3.9	

proposed in the literature has been obtained by indirect methods. It is a great challenge to the organic chemist and the chemical physicist to develop methods that allow a direct structure determination of the ions in flight.

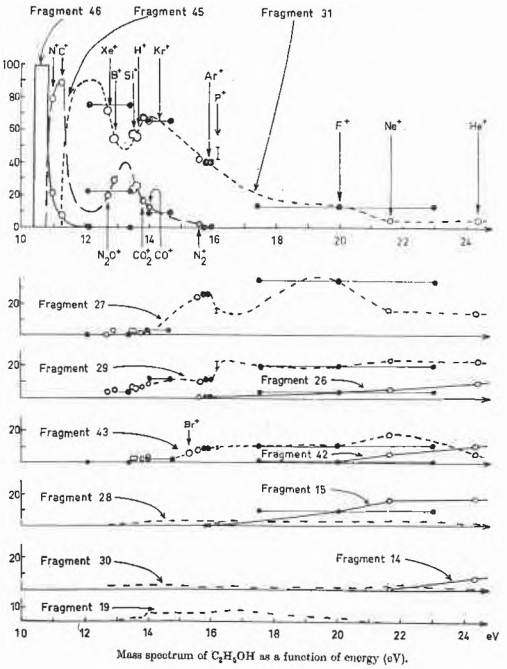


Fig. 23. Mass spectrum of ethanol as a function of the absorbed energy. From ref. [25]

FIELD and MUNSON[26, 27] have recently described a somewhat different charge exchange technique which involves charge exchange from more than one ion species but in comparison with LINDHOLM's method has the advantage of being comparatively simple from the experimental point of view. The technique, which has been called chemical ionization mass spectrometry, involves obtaining the mass spectrum of the compound under study in the presence of large amount of another gas (at a pressure up to 2 Torr) in the ion source. If, for instance, methane is used as the high pressure component, primary ionization by electron impact occurs practically only for methane molecules. Ions such as $[CH_5]^+$ and $[C_2H_5]^+$ are formed (cf. Table 2) which do not react further with methane molecules but are capable of charge exchange ionization of the molecules of the compound under study. The resulting mass spectrum has often an appearance that is quite different from that obtained by electron impact. It is of special interest that compounds which give no molecule-ion peak in the electron impact spectrum may show large peaks at m/e = M−1, M, or M+1 in the chemical ionization spectra. The ions of m/e = (M−1) and (M+1) caused by loss of hydride ion and proton transfer, respectively, from the molecule-ion are called "quasi molecular ions" by MUNSON and FIELD. Fig. 25 a shows the electron impact (70 eV) and the methane chemical ionization spectra of *n*-decanol-1. The C. I. spectrum shows a distinct peak

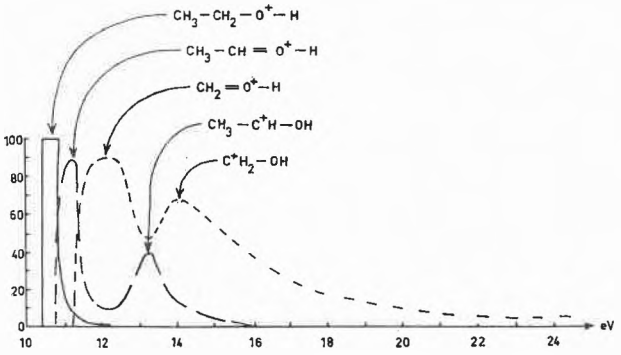


Fig. 24. Structure of certain ions in the mass spectrum of ethanol deduced by VON KOCH and LINDHOLM[25]

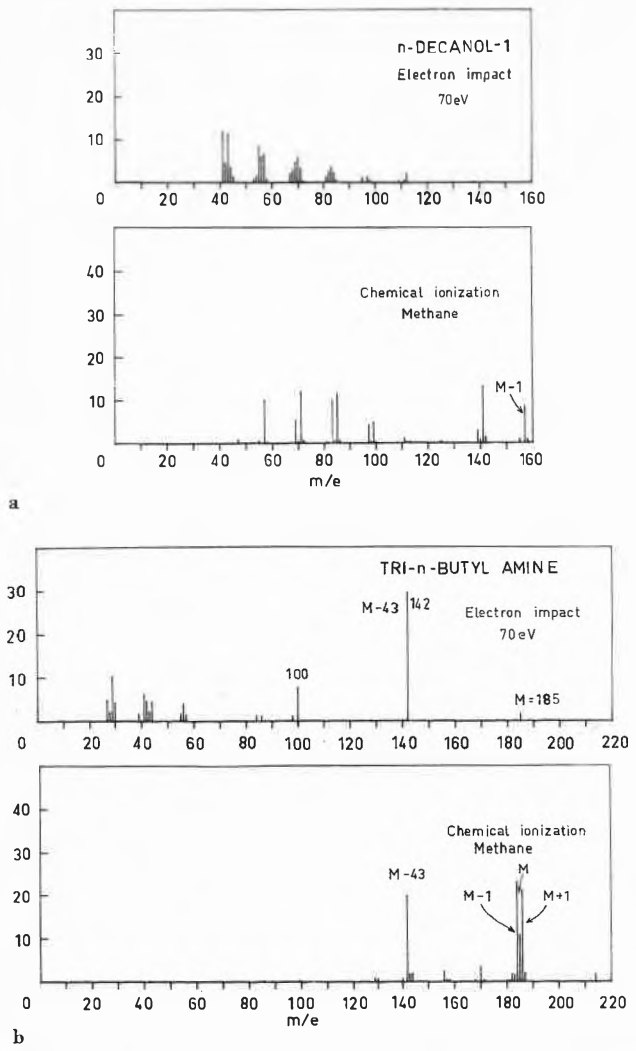
Table 2. Mass spectrum of methane at a pressure of 1.0 Torr and partial mass spectrum of methane at 1.0 Torr with 0.1% *n*-hexadecane added. From data of MUNSON and FIELD, kindly submitted prior to publication

m/e	Ion	Chemical Ionization Intensities (Chart Div.) CH_4 Only	$CH_4 + nC_{16}H_{34}$
17	CH_5^+	25,900	22,500
18		340	265
19	H_3O^+	1,470	1,040
28	$C_2H_4^+$	1,900	1,570
29	$C_2H_5^+$	25,200	21,700
30		625	550
31		292	190
39	$C_3H_3^+$	80	80
40	$C_3H_4^+$	145	120
41	$C_3H_5^+$	5,070 [a]	4,300
42		255	210
43	$C_3H_7^+$	3,300	2,240
44		115	90
55	$C_4H_7^+$	38	53
57	$C_4H_9^+$	1,560 [a]	1,530
58		90	94
69	$C_5H_9^+$		9
70	$C_5H_{10}^+$		8
71	$C_5H_{11}^+$		430
72			29
83	$C_6H_{11}^+$		10
85	$C_6H_{13}^+$		370
86			30
97	$C_7H_{13}^+$		9
98	$C_7H_{14}^+$		17
99	$C_7H_{15}^+$		275
100			22
111	$C_8H_{15}^+$		9

[a] The methane used was slightly contaminated with isobutane.

[26] F. H. FIELD and M. S. B. MUNSON, *J. Amer. Chem. Soc. 87* (1965) 3294.

[27] M. S. B. MUNSON and F. H. FIELD, *J. Amer. Chem. Soc, 87* (1965) 3294, 4242.



at m/e = 157 = M−1. The C.I. mass spectrum of tri-*n*-butyl amine in Fig. 25b shows a cluster of peaks at m/e = M−1, M, and M+1. In this case the electron impact spectrum shows a peak due to the molecule-ion. The electron impact mass spectrum of *n*-heptyl propionate (Fig. 25c) gives no information about the molecular weight whereas the C.I. mass spectrum shows a peak at m/e = M+1. It seems evident that mass spectra produced by ion-molecule interactions will become increasingly important in the future.

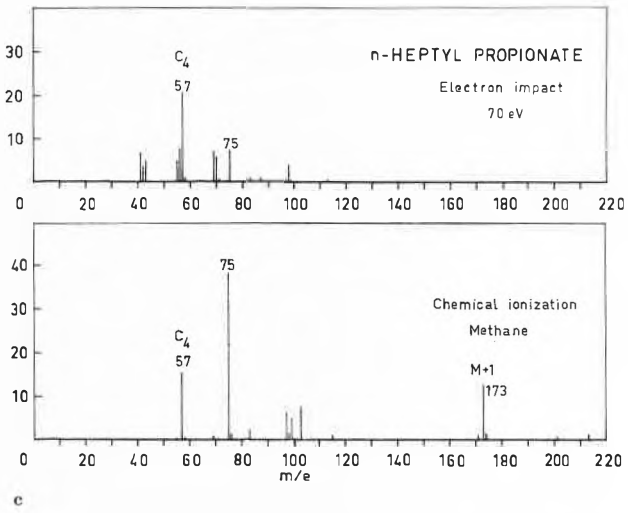


Fig. 25. Comparison between electron impact and chemical ionization (methane) mass spectra. a) *n*-decanol-1; b) tri-*n*-butylamine; c) *n*-heptyl propionate. Constructed from data kindly furnished by Dr. Frank Field